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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : C07H 21/00</p>	<p>A2</p>	<p>(11) International Publication Number: <b>WO 99/16780</b> (43) International Publication Date: 8 April 1999 (08.04.99)</p>
<p>(21) International Application Number: PCT/BE98/00141 (22) International Filing Date: 28 September 1998 (28.09.98) (30) Priority Data: 97870146.4 26 September 1997 (26.09.97) EP (71) Applicants (for all designated States except US): UNIVERSITE CATHOLIQUE DE LOUVAIN [BE/BE]; Halles Universitaires, Place de l'Université 1, B-1348 Louvain-la-Neuve (BE). MINISTERE DE LA DEFENSE NATIONALE [BE/BE]; Etat Major Général, JSM - R &amp; T, Quartier Reine Elisabeth, Rue d'Evere 1, B-1140 Bruxelles (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): VANNUFFEL, Pascal [BE/BE]; Rue de la Basse Egypte 138, B-7133 Buvinnes (BE). GALA, Jean-Luc [BE/BE]; Rue Grand Chemin Communal 6, B-5380 Fernelmont (BE). (74) Agents: VAN MALDEREN, Eric et al.; Office Van Malderen, Place Reine Fabiola 6/1, B-1083 Bruxelles (BE).</p>		<p>(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published Without international search report and to be republished upon receipt of that report.</p>
<p>(54) Title: GENETIC SEQUENCES, DIAGNOSTIC AND/OR QUANTIFICATION METHODS AND DEVICES FOR THE IDENTIFICATION OF <i>STAPHYLOCOCCI</i> STRAINS  (57) Abstract  The present invention is related to oligonucleotides for the specific identification of <i>Staphylococci</i> species which nucleotide sequence has between 15 and 350 base pairs, preferably between 15 and 45 base pairs, obtained from the "consensus" <i>femA</i> nucleotide sequence (CNS) of the figure or its complementary strand. The present invention is also related to a method and a diagnostic device using said oligonucleotide for the identification of various types of <i>Staphylococci</i> species strains.</p>		

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GENETIC SEQUENCES, DIAGNOSTIC AND/OR QUANTIFICATION METHODS  
10 AND DEVICES FOR THE IDENTIFICATION OF STAPHYLOCOCCI STRAINS

Field of the invention

The present invention refers to new genetic sequences, diagnostic and/or quantification methods and  
15 devices using said sequences for the identification of various types of Staphylococci strains as well as the therapeutical aspects of said genetic sequences.

Background of the invention

20 Increasing incidence of nosocomial infections by multiresistant bacteria (even to antibiotics like vancomycin) is a world-wide concern. Methicillin-resistant coagulase-negative Staphylococci (MR-CNS) and *S. aureus* (MRSA) express a high level cross-resistance to all  $\beta$ -  
25 lactam antibiotics (Ryffel et al. (1990), Refsahl et al. (1992)). They have an additional low-affinity penicillin-building protein, PBP2a (PBP2'), encoded by the *mecA* gene. The *mecA* determinant is found in all multiresistant staphylococcal species (Chackbart et al. (1989), Suzuki et al. (1992), Vannuffel et al. (1995)) and is highly  
30 conserved among the different species (Ryffel et al. (1990)).

Several other chromosomal sites, in which transposon inactivation reduces the level of  $\beta$ -lactam resistance, have been identified in *S. aureus* (SA) (Hiramatsu (1992), Berger-Bächi et al. (1992), de Lancastre  
5 et al. (1994)). The appropriate functioning of these regulator genes rather than the quantity of PBP2a determines the minimal inhibitory concentration value and homogeneous expression of resistance of staphylococcal isolates (Ryffel et al. (1994), de Lancastre et al.  
10 (1994)).

The *femA-femB* operon, initially identified in *S. aureus*, is one of those genetic factors essential for methicillin resistance (Berger-Bächi et al. (1989)). It is involved in the formation of the characteristic  
15 pentaglycine side chain of the SA peptidoglycan (Stranden et al. (1997)). Unlike other regulatory genes, *femA* was shown to retain a strong conservation over time in clinical isolates of MRSA, hence confirming its key role in cell wall metabolism and methicillin resistance (Hurlimann-Dalel  
20 et al. (1992)). In contrast to *mecA*, *femA-femB* is present both in the genome of resistant and susceptible SA strains (Unal et al. (1992), Vannuffel et al. (1995)).

Often, identification of the *Staphylococci* is limited to a rapid screening test for *S. aureus*, and non-*S.*  
25 *aureus* isolates are simply reported as coagulase-negative *Staphylococci*. In fact, these bacteria isolates include a variety of species and many different strains (Kleeman et al. (1993)). There is little epidemiological information related to the acquisition and spread of these organisms.  
30 This is potentially due to the lack of an easy and accurate way to identify species and to provide clinically timely informations.

Several molecular assays designed for detecting *femA* in SA failed to amplify an homologous sequence in coagulase-negative *Staphylococci* (Kizaki et al. (1994), Vannuffel et al. (1995)). Nevertheless, low-  
5 stringency heterologous hybridisation analysis suggested the presence of such a structurally related gene in *S. epidermidis* (SE) (Unal et al. (1992)).

These data were followed by complete identification and sequence analysis of the *femA* and *femB*  
10 open reading frames in *S. epidermidis* (Alborn et al. (1996)). Intra- and interspecies relatedness of these genes and conservation of genomic organisation are therefore consistent with gene duplication of one of these genes in an ancestral organism and the possibility of *femA*  
15 phylogenetic conservation in all staphylococcal species (Alborn et al. (1996)).

The complete genetic sequence of the *femA* gene de *S. epidermidis*, the protein encoded by the *femA* gene (*FemA*) and vectors and micro-organisms comprising  
20 genes encoding the *FemA* protein are described in the US patent 5,587,307.

#### Aims of the invention

The present invention aims to provide new  
25 genetic sequences, methods and devices for the improvement of the identification and/or the quantification of various types of *Staphylococci* strains through their *femA*-like determinants, which allow by a rapid screening their epidemiological study.

30 Another aim of the invention is to identify similar genetic sequences which may exist in known or not

known *Staphylococci* species or other gram-positive bacterial strains.

A last aim of the present invention is to provide new sequences encoding *femA* proteins of  
5 *Staphylococci* species, their *femA* proteins, vector(s) comprising said nucleotide sequences and cell (s) transformed by said vector(s) for possible therapeutical applications.

#### 10 Summary of the invention

The Inventors have identified new DNA and amino acid sequences from new strains of *Staphylococcus hominis*, *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus*. Said new nucleotide sequences allow an  
15 alignment of these new sequences with the *femA* gene from *Staphylococci* previously described (*S. aureus*, *S. epidermidis* and *S. saprophyticus*). By the alignment of more than 2 sequences, preferably more than 4 sequences, the Inventors have identified for the first time a consensus  
20 *femA* sequence useful for molecular genotyping of different *Staphylococci* species which was not possible previously, when only few *femA* sequences of *Staphylococci* strains were known.

Therefore, a first aspect of the present  
25 invention is related to the "consensus" nucleotide sequence as represented in the enclosed Figure 3. With said "consensus" nucleotide sequence, the Inventors were able to provide oligonucleotides (such as primers or probes) which can be used for the genetic amplification, the  
30 identification and/or quantification of various *femA* sequences which are specific of known or unknown *Staphylococci* species.

The *femA* sequence is known to be involved with the biosynthesis of glycin-containing cross-bridges of the peptidoglycan and the peptidoglycan organisation is also known to be well conserved among various *Staphylococci* species and possibly among other gram-positive bacteria.

Therefore, it is also possible to use the new "consensus" *femA* sequence and said new oligonucleotides extrapolated from the alignment of the sequences presented in Figure 3, for the molecular genotyping of other *Staphylococci* species and possibly other gram-positive bacteria. It is also known that the *femA* sequence is similar to the *femB* sequence. Therefore, these oligonucleotides could also be used for the molecular genotyping of *femB* genes of different *Staphylococci* species or other gram-positive bacteria.

Another aspect of the present invention concerns the possible therapeutical uses of new *femA* nucleotide sequences isolated from the strains *S. hominis*, *S. saprophyticus*, *S. haemolyticus*, *S. lugdunensis*, *S. xylosus*, *S. capitis*, *S. schleiferi* and *S. sciuri* having a nucleotide or amino acid sequence which presents more than 85%, preferably more than 90% homology or 100% homology with the genetic sequences presented in the Figures 6 to 13, their complementary strand and functional variants thereof. Functional variants of said amino acid sequences are peptides which contain one or more modifications to the primary amino acids sequence and retain the activity of the complete and wild type *femA* molecule. Variants of the peptide are obtained by nucleotidic sequences which differ from the above-identified described sequences by a degeneration of their genetic code or are sequences which hybridise with said sequences or their complementary

strand, preferably under stringent conditions such as the ones described in the document Sambrook et al., §§ 9.47-9.51 in *Molecular Cloning : A Laboratory Manual*, Cold Spring Harbor, Laboratory Press, Cold Spring Harbor, New York (1989).

A further aspect of the present invention concerns the recombinant vector (i.e. constructions into which the sequence of the invention may be inserted for transport in different genetic environments and for expression in a host cell, such as a phagemide, a virus, a plasmid, a cationic vesicle, a liposome, etc.) comprising said nucleotide sequences and their complementary strands, or the corresponding RNA sequences, possibly linked to one or more regulatory sequences or markers (resistance to antibiotics, enzyme coding sequences, ...) active into a cell.

Similarly, the nucleic acid sequence according to the invention may be obtained by synthetic methodology well known by the person skilled in the art, such as the one described by Brown et al. ("Method of Enzymology", Acad. Press, New-York, No. 68 pp. 109-151 (1979)) or by conventional DNA synthesising apparatus such as the applied biosystem model 380A or 380B DNA synthesiser.

Other aspects of the present invention concern the recombinant host (prokaryotic) cell transformed by said vector and the purified (possibly recombinant) proteins or peptides encoded by said nucleic acid sequences, possibly linked to a carrier molecule such as BSA and obtained by said cells. Said recombinant proteins or peptides could be obtained by genetic engineering or could be obtained by synthesis (see US patent 5,587,307



incorporated herein by reference) and may comprise residues enhancing their stability (resistance to hydrolysis by proteases, etc.) such as the one described by Nachman et al. (*Regul. Pept.* Vol. 57, pp. 359-370 (1995)).

5                   A preferred vector for expression in a *E. coli* host cell is derived from the *E. coli* plasmid pET-11A available from Novagen Inc. (Catalogue No. 69436-A). The transformation technique used with the above-identified vector has been described in the US Patent 5587307.

10                   A further aspect of the present invention concerns the inhibitor (used to possibly treat (with addition of antibiotics) antibiotics resistance bacteria) directed against said proteins, peptides or nucleic acid molecules. Advantageously, said inhibitor is a antibody,  
15 preferably a monoclonal antibody, or an antisense nucleotide molecule, such as a ribozyme, which could be present in a vector in order to block the expression of said *femA* nucleotide sequences.

                  A last aspect of the present invention  
20 concerns the pharmaceutical composition, preferably a vaccine, against *Staphylococci* infections in an animal, including a human, comprising a pharmaceutically acceptable carrier and a sufficient amount of an active compound selected from the group consisting of said nucleic acid  
25 molecules, vectors, recombinant host cells transformed by said vector(s), inhibitors (directed against said proteins, peptides or nucleic acid molecules) and a mixture thereof.

                  Another aspect of the present invention concerns oligonucleotides which are (DNA) sequences having  
30 between 15 and 350 base pairs, preferably between 17 and 250 base pairs (such as primers or probes) obtained from the consensus sequence of Figure 3 or its complementary

strand. Preferably, said oligonucleotides are primers having between 15 and 45 base pairs, more preferably between 17 and 25 base pairs.

According to a first embodiment of the present invention, said oligonucleotide is a primer having between 15 and 45 base pairs, which presents more than 60%, advantageously more than 70%, preferably more than 80%, more specifically more than 90% homology with (fragments of) the "consensus" *femA* nucleotide sequence (CNS) identified in the Figure 3. :

Therefore, the oligonucleotides according to the invention are new sequences or preferred fragments of known sequences of *S. aureus*, *S. epidermidis* or *S. simulans* but not the complete wild type known *femA* nucleotide sequence.

Preferably, the oligonucleotide according to the invention is selected from the group consisting of the following nucleotide sequences :

- ANAATGAANTTTACNAATTTNACNGCNANAGANTT
- 20 and more particularly *femS1* TAATGAAGTTTACAAAATTT or *femS2* TAATGAAGTTTACNAAATTT
- ATGNCNNANAGNCATTTNACNCANA
- and more particularly *femU1* ("universal" sequence sense of the multiplex PCR): TGCCATATAGTCATTTACGC
- 25 - TAGTNGGNATNAANAANAANNATAANGANGTNATTGC
- GTNCCNGTNATGAAANTNTTNAANTANTTTTATTC
- AATGCNGGNNANGATTGG
- GNAANNGNAANACNAAAAAGTNNANAANAATGGNGTNAAAGT
- and more particularly *fsq1S* (et 1AS) :
- 30 AAAAAGTTCAAAAATGG and *fsq2S* (and 2AS) :
- AAAAAGTACAAAATGG
- AAGANGANNTNCCNATNTTNGNTCATTNATGGANGATAC

- TATATNNANTTTGATGANTA
- AANGANATNGANAAANGNCCNGANAANAAAAA
- and more particularly *fsq3S* (and 3AS) :
- AAAGATATTGAAAAACGA, *fsq4S* (and 4AS) :
- 5 AAAGATATTGAAAAGAGACC, *fsq5S* (and 5AS) :
- AAAGATATCGAGAAAGAC and *fsq6S* (and 6AS) :
- AAAGACATCGACAAGCGT.
- ANCATGGNAANGAATTACCNAT
- and more particularly *fem1* (primer for the production
- 10 of a probe and of marked amplicons for reverse
- hybridisation experiment) : GAACATGGTAATGAATTAC
- AATCCNTNTGAAGTNGTNTANTANGCNGGTGG
- AGNTATGCNNTNCAATGGNNNATGATTAANTATGC
- TTTANNGANGANGCNGAAGATGNNGGNGTNTNAANTTNAAAAA
- 15 and more particularly *fem3bio* (primer for the
- production of a probe and of marked amplicons for
- reverse hybridisation experiment) :
- TTTACTGAAGATGCTGAAGA
- GTTGGNGANTTNNTNAAACC
- 20 and more particularly *fem2* (primer for the production
- of a probe and of marked amplicons for reverse
- hybridisation experiment) : GTTGGTGACTTTATTAAACC
- ATGAAATTTACAGAGTTAA (= *femAS1*)

25 Said primer(s) will be designated hereafter  
as "universal primer(s)".

A further aspect of the present invention  
concerns the oligonucleotide being either a primer or a  
probe as above-described, having between 15 and 350 base  
30 pairs, preferably between 17 and 250 base pairs, or a  
primer having between 15 and 45 base pairs, more preferably  
between 17 and 25 base pairs, which will be designated

hereafter as "specific primer(s)", having a nucleotide sequence which presents less than 50%, advantageously less than 40%, preferably less than 30%, more specifically less than 20% homology with (fragments of) the "consensus" *femA* 5 nucleotide sequence (CNS) identified in the Figure 3 and with another *femA* nucleotide sequence specific for other *Staphylococci* strains.

Advantageously, said "specific primer" is selected from the group consisting of the following 10 nucleotide sequences :

- ACAGCAGATGACATCATT
- TAATGAAAGAAATGTGCTTA
- ACACAACCTTCAATTAGAAC
- AGTATTAGCAAATGCGG
- 15 - ATGCATATTTTCCGTAA
- CAGCAGATGACATCATT
- CATCTAAAGATATATTAAATGGA
- AGTATTAGCAAATGCGGGTCAC
- CAACACAACCTTCAATTAGAA

20

The oligonucleotides according to the invention are selected according to their physiochemical properties in order to avoid cross-hybridisation between themselves. Said primers are not complementary to each 25 other and they contain a similar percentage of bases GC.

Said oligonucleotides are used in an identification and/or quantification method of one or more *Staphylococcus* species and possibly other gram-positive bacteria.

30

Therefore, another aspect of the present invention is related to an identification and/or

quantification method of a *Staphylococci* species which may present resistance to one or more antibiotic(s), and is possibly combined with a method for the identification of a resistance to antibiotics, especially  $\beta$ -lactam antibiotics, (for instance through the identification of a variant of the *mecA* gene as described by Vannuffel et al. (1998)).

The method for the detection, the identification and/or the quantification of a bacteria, preferably a staphylococcal species, comprises the steps of :

- obtaining a nucleotide sequence from said bacteria present in a sample, preferably a biological body sample obtained from a patient such as blood, serum, dialyse liquid or cerebrospinal liquid, or from any other bacteriological growth medium,
- possibly purifying said nucleotide sequence from possible contaminants,
- possibly amplifying by known genetic amplification techniques said nucleotide sequence with one or more universal oligonucleotide(s) (universal primer(s)) according to the invention, and
- identifying the specific gram-positive bacteria species, preferably the specific *Staphylococci* species :
  - by a comparative measure of the length of the (possibly amplified) nucleotide sequence or
  - by reverse hybridisation of the (possibly amplified) nucleotide sequence with one or more specific oligonucleotide(s) (specific probe(s) or primer(s)) according to the invention which are specific of said bacteria, said oligonucleotide(s) being preferably immobilised on a solid support.

The comparative measure of the length of a possibly amplified nucleotide sequences can be performed by the analysis of their migration (compared with a known ladder) upon an electrophoresis gel.

5                    Preferably, the genetic amplification technique is selected from the group consisting of PCR (US patent 4,965,188), LCR (Landgren et al., *Sciences*, 241, pp. 1077-1080 (1988)), NASBA (Kievits et al., *J. Virol. Methods*, 35, pp. 273-286 (1991)), CPR (patent WO95/14106)  
10 or ICR.

The specific detection of the possibly amplified nucleotide sequences can be obtained by the person skilled in the art by using known specific gel electrophoresis techniques, in situ hybridisation,  
15 hybridisation on solid support, in solution, on dot blot, by Northern blot or Southern blot hybridisation, etc.

Advantageously, the probes which are specific of the bacteria are immobilised on a solid support according to the method described in the international  
20 patent application WO98/11253 incorporated herein by reference.

Said specific oligonucleotides (probes or "elongated" primers) have a length comprised between 50 and 350 base pairs, preferably between 120 and 250 base pairs,  
25 and are fixed to the solid support by a terminal 5' phosphate upon an amine function of the solid support by carbodiimide reaction (as described in the document WO98/11253 incorporated herein by reference).

The solid support can be selected from the  
30 group consisting of cellulose or nylon filters, plastic supports such as 96-well microtiter plates, microbeads,

preferably magnetic microbeads, or any other support suitable for the fixation of a nucleotide sequence.

The method according to the invention can be advantageously combined with another specific detection  
5 step of a possible resistance to antibiotics, especially  $\beta$ -lactam antibiotics (for instance through the identification by the above-described technique of variants of the *mecA* gene as described by Vannuffel et al. (1998)).

The present invention concerns also a  
10 diagnostic and/or quantification device or kit for the identification and/or the quantification of a *Staphylococcus* species or other gram-positive bacteria, comprising the oligonucleotides according to the invention and possibly all the media necessary for the identification  
15 of a (possibly amplified) nucleotide sequence of said bacteria through any one of the above-described methods.

Advantageously, the method and device according to the invention are adapted for the quantification of said *Staphylococci* strains by the use of  
20 a "internal or external standard sequence", preferably the one described in the patent application WO98/11253 incorporated herein by reference.

Therefore, according to a first embodiment of the present invention, the nucleic acid sequence from a  
25 *Staphylococcus* species, for instance *Staphylococcus aureus*, is amplified by a "universal primer" and by a "specific primer" which is specific for *S. aureus*. The identification of *S. aureus* will be obtained upon an agarose electrophoresis gel wherein the amplified nucleotide  
30 sequence (shorter than the amplified nucleotide sequence of another *Staphylococci* species such as *S. epidermidis*) and identified by the use of a comparative ladder.

According to another embodiment of the present invention, a *Staphylococcus* species (such as *S. aureus*) is identified by reverse hybridisation of the amplified nucleotide sequence with a probe which is  
5 specific of said bacteria and which is immobilised on a solid support such as filter.

The present invention will be described in details in the following non-limiting examples, in reference to the Figures described hereafter.

10

Short description of the drawings

The Figure 1 represents 5 partially overlapping fragments of the *femA* genes from *S. hominis*, *S. saprophyticus* and *S. haemolyticus* obtained  
15 by PCR amplification.

The Figure 2 represents the alignment of the nucleotide sequences of *femA* genes from *S. hominis*, *S. saprophyticus*, *S. aureus*, *S. epidermidis* and *S. haemolyticus*.

20 The Figure 3 represents the consensus sequence according to the invention.

The Figure 4 represents the result of differential diagnosis between different strains of *Staphylococci* by reverse hybridisation.

25 The Figure 5 represents amplification of CNS species under universal conditions.

Figures 6 to 13 represent the complete *femA* wild type genetic sequence of the strains *S. hominis*, *S. saprophyticus*, *S. haemolyticus*, *S. lugdunensis*, *S. xylosus*, *S. capitis*, *S. schleiferi* and *S. sciuri*.  
30



### Examples

#### Example 1 : Sequencing strategy

Fragments of the *femA* genes from *S. hominis* and *S. saprophyticus* have been obtained by PCR amplification, in low stringency annealing conditions. Primers used for amplification are matching the potentially conserved regions and have been designed according to sequences homologies between *S. aureus*, *S. saprophyticus* and *S. epidermidis* *femA* nucleotide sequences. For both *S. hominis* and *S. saprophyticus* species, 5 partially overlapping fragments have been synthesised allowing the sequencing of the entire *femA* genes (Fig. 1).

#### Example 2 : Identification of a consensus sequence

Alignment of the nucleotide sequences of *femA* genes from *S. hominis* and *S. saprophyticus* as well as with *femA* genes sequenced to date from *S. aureus* (GenBank accession number M23918), *S. epidermidis* (GenBank accession number U23713) and *S. haemolyticus* is presented in Fig. 3 and has allowed to propose a "consensus" *femA* nucleotide sequence (CNS) whose genomic organisation displays highly conserved regions flanked by variable ones. On this basis, interspecies phylogenetic variations could be exploited to design genotyping strategies for species-specific identification of *Staphylococci*. The "consensus" sequence is therefore a powerful molecular tool for specific diagnostic of staphylococcal infections.

#### Example 3 : Sequencing of other staphylococcal *femA* genes

The consensus sequence can be exploited for designing universal primers allowing the production, under permissive annealing conditions, of overlapping PCR

products whose sequencing will identify the entire *femA* sequence.

Example 4 : Differential diagnosis between *S. aureus*, *S. epidermidis*, *S. hominis* and *S. saprophyticus* by reverse hybridisation

The Inventors have set up a reverse hybridisation assay for rapid and combined identification of the most clinically relevant *Staphylococci* species, and their *mecA* status. Two sets of primers, chosen in a conserved domain of the consensus sequence (*bioU1-bioU3* and *fem1-fem3bio*), amplifying a 286 and bio-220 bp fragments, respectively) were synthesised. Species-specificity of *femA* amplicons was insured by the genomic variability between the conserved regions. *FemA* probes were immobilised on nylon strips. Hybridisation was performed with biotinylated *femA* PCR fragments from the strain of interest. The strategy was first assessed with ATCC strains (*S. aureus*, *S. epidermidis*, *S. hominis* and *S. saprophyticus*) (Fig. 4). Specificity was identified by standard methods. Accuracy was 100% for species identification.

Example 5 : Differential diagnosis between staphylococcal species

This assay is able to identify any staphylococcal species if following requirements are fulfilled :

- primers *fem1*, *fem2* and *fem3bio* are universal for *Staphylococci*;
- there is a wide enough phylogenetic variation between any CNS species to promote a specific hybridisation.

The first requirement is fulfilled for, i.e., *S. haemolyticus*, *S. capitis*, *S. cohnii*, *S. xylosum*, *S. simulans*, *S. lugdunensis*, *S. schleiferi* and *S. warneri* strains (Fig. 5).

5

Example 6 : Multiplex amplification of *femA* and *mecA* genetic determinants for a molecular diagnosis of a specific staphylococcal infection

A total of 48 patients treated in 4  
10 contiguous intensive cares units were included in the study. Endotracheal aspirates (ETA) were collected from the patients and submitted to the multiplex PCR analysis according to the technique described by Vannuffel et al. (1995). Clinical specimens were homogenised in 5 ml of TE  
15 buffer (20 mM TRIS HCl, pH 8.0, 10 mM EDTA) containing 2% (w/v) SDS.

The homogenate (1.5 ml) was then centrifuged for 5 minutes at 7500 xg. The cellular pellet was washed once with TE buffer lysed in the presence of 1% (v/v)  
20 Triton X-100 and 50 µg of lysostaphin (Sigma) and incubated for 15 minutes at 37 °C. Lysis was completed by adding 100 µg of proteinase K (Boehringer). The lysate was incubated for another 5 minutes at 55 °C and 5 minutes at 95 °C, and centrifuged at 4000 xg for 5 minutes.

25 In order to purify bacterial DNA, 200 µl of supernatant were then filtered on a Macherey-Nagel Nucleospin C+T® column and eluted with 200 µl sterile H<sub>2</sub>O. Two different amounts of DNA suspension (2 µl and 200 µl) were submitted to multiplex PCR amplification with the  
30 primers 5'-TGGCTATCGTGTCACAATCG-3' and 5'-

CTGGAACCTTGTTGAGCAGAG-3' for *mecA* and the above-described primers for *femA*, yielding different fragments.

*femA* and *mecA* signals were found in specimens containing either susceptible *S. aureus* (n = 10) and 5 methycillin-resistant coagulase-negative *Staphylococci* (n = 6) respectively. On the other hand, no signal was obtained from ETA gram-negative bacteria (n = 5) as well as MS-CNS (n = 6) and from 5 ETA containing normal pharyngeal flora.

10 This multiplex, PCR strategy for detecting *Staphylococci* in ETA was completed in less than 6 hours either on the day of the samples' collection. This is an advantage with respect to the time required to conventional identification and susceptibility tests (48 to 72 hours).

15

Example 7 : Amplification, cloning and sequencing of other *femA* genes

Two primers were selected among the conserved parts of the consensus sequence for the amplification of 20 the *femA* gene.

These primers are *femS1*, *femS2* and *femAS1* (anti-sense primer). ADN from strains of *Staphylococcus hominis*, *saprophyticus*, *haemolyticus*, *lugdunensis*, *schleiferi*, *sciuri*, *xylosus*, *simulans*, *capitis*, *gallinarum*, 25 *cohnii* and *warneri* were amplified from said primers and amplification fragments were cloned in the vector pCR®-XLTOPO and introduced by electroporation in *E. coli* cells TOP10 (TOPO XL PCR Cloning Kit®, Invitrogen, Carlsbad, CA).

30 Amplified fragments of strain *S. lugdunensis*, *schleiferi*, *sciuri*, *xylosus*, and *capitis* were sequenced by Taq Dye Deoxy Terminator Cycle® sequencing on a ABI 277 DNA

sequencer® (PE Applied Biosystems, Foster City, CA) by the following primers :

*femS1* or *femS2* or *femAS1*

*fsq1S* and *fsq1AS*

5 *fsq2S* and *fsq2AS*

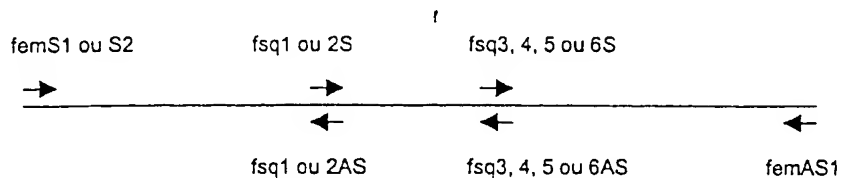
*fsq3S* and *fsq3AS*

*fsq4S* and *fsq4AS*

*fsq5S* and *fsq5AS*

*fsq6S* and *fsq6AS*

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CLAIMS

1. Oligonucleotide for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 45 base pairs, preferably between 15 and 25 base pairs, and which presents more than 60% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

2. Oligonucleotide according to claim 1 for the specific identification of *Staphylococci* species, which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 70% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

3. Oligonucleotide according to claim 1 or 2 for the specific identification of *Staphylococci* species, which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 80% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

4. Oligonucleotide according to any of the claims 1 to 3 for the specific identification of *Staphylococci* species, which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 90% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

5. Oligonucleotide according to any of the preceding claims, which is selected from the group consisting of the following nucleotide sequences :

- ANAATGAANTTTACNAATTTNACNGCNANAGANTT

and more particularly TAATGAAGTTTACAAAATTT or  
TAATGAAGTTTACNAAATTT

- ATGNCNNANAGNCATTTNACNCANA  
and more particularly TGCCATATAGTCATTTACGC
- TAGTNGGNATNAANAANAANNATAANGANGTNATTGC
- GTNCCNGTNATGAAANTNTTNAANTANTTTTATTC
- 5 - AATGCNGGNNANGATTGG
- GNAANNGNAANACNAAAAAGTNNANAANAATGGNGTNAAAGT  
and more particularly AAAAAGTTCAAAAAATGG and  
AAAAAGTACAAAAATGG
- AAGANGANNNTNCCNATNTTNGNTCATTNATGGANGATAC
- 10 - TATATNNANTTTGATGANTA ,
- AANGANATNGANAAANGNCCNGANAANAAAAA  
and more particularly AAAGATATTGAAAAACGA,  
AAAGATATTGAAAAGAGACC, AAAGATATCGAGAAAGAC and  
AAAGACATCGACAAGCGT.
- 15 - ANCATGGNAANGAATTACCNAT  
and more particularly GAACATGGTAATGAATTAC
- AATCCNTNTGAAGTNGTNTANTANGCNGGTGG
- AGNTATGCNNTNCAATGGNNNATGATTAANTATGC
- TTTANNGANGANGCNGAAGATGNNGGNGTNNTNAANTTNAAAAA
- 20 and more particularly TTTACTGAAGATGCTGAAGA
- GTTGGNGANTTNNTNAAACC  
and more particularly GTTGGTGACTTTATTAAACC
- ATGAAATTTACAGAGTTAA

6. Oligonucleotide for the specific  
25 identification of *Staphylococci* species which nucleotide  
sequence has between 15 and 350 base pairs, preferably  
between 17 and 250 base pairs, and which presents less than  
50% homology with the "consensus" *femA* nucleotide sequence  
(CNS) of Fig. 3.

30 7. Oligonucleotide according to claim 6 for  
the specific identification of *Staphylococci* species which  
nucleotide sequence has between 15 and 350 base pairs,



preferably between 17 and 250 base pairs, and which presents less than 40% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

8. Oligonucleotide according to claim 6 or 7  
5 for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 30% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

10 9. Oligonucleotide according to any of the claims 6 to 8 for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 20% homology with the  
15 "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

10. Oligonucleotide according to claim 6, being a primer which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs.

11. Oligonucleotide according to claim 10,  
20 which is selected from the group consisting of the following nucleotide sequences :

- ACAGCAGATGACATCATT
- TAATGAAAGAAATGTGCTTA
- ACACAACCTTCAATTAGAAC
- 25 - AGTATTAGCAAATGCGG
- ATGCATATTTTCCGTAA
- CAGCAGATGACATCATT
- CATCTAAAGATATATTAAATGGA
- AGTATTAGCAAATGCGGGTCAC
- 30 - CAACACAACCTTCAATTAGAA

12. Identification and/or quantification method of a *Staphylococci* species, which may present resistance to antibiotics and which is present in a sample, said method comprising the steps of :

- 5 - obtaining a nucleotide sequence from a *Staphylococci* species present in the sample,
  - amplifying said nucleotide sequence with one or more oligonucleotide(s) according to the claims 1 to 8, and
  - identifying and possibly quantifying the specific
- 10 *Staphylococci* species :
- by reverse hybridisation of the amplified nucleotide sequence with one or more oligonucleotide(s) according to the claims 9 to 11 which is (are) specific of said *Staphylococci*
  - 15 species and is (are) immobilised on a solid support or
  - by a comparative measure of the length of the amplified nucleotide sequence.

13. Diagnostic device for the identification

20 of *Staphylococci* species comprising the oligonucleotide according to any of the preceding claims 1 to 11 and possibly all the media necessary for the identification of an amplified sequence of said *Staphylococci* species through any one of the methods selected from the group consisting

25 of in situ hybridisation, hybridisation on a solid support, in solution on dot blot, Northern blot, Southern blot, probe hybridisation by the use of an isotopic or non-isotopic label, genetic amplification or a mixture thereof.

14. *femA* genetic sequence which presents more

30 than 90% homology with a nucleotide or amino acid sequence selected from the group consisting of the nucleotide or

amino acid sequences represented in the enclosed Fig. 6 to 13.

15. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 6.

5 16. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 6.

17. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 7.

18. Genetic sequence according to claim 14, 10 being the amino acid sequence, of Fig. 7.

19. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 8.

20. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 8.

15 21. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 9.

22. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 9.

23. Genetic sequence according to claim 14, 20 being the nucleotide sequence of Fig. 10.

24. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 10.

25. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 11.

25 26. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 11.

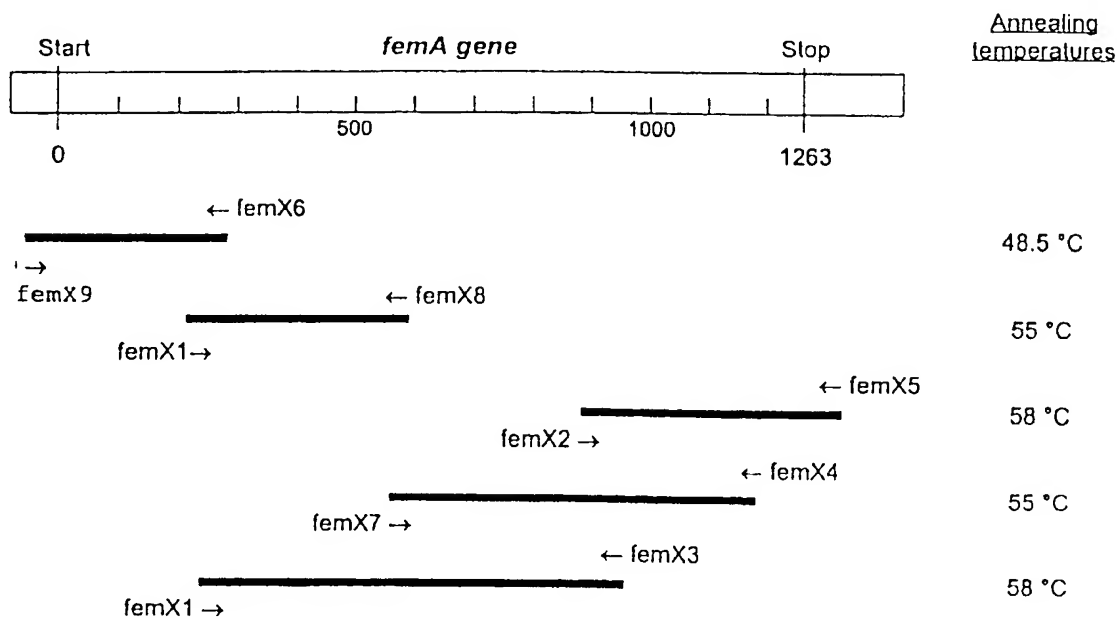
27. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 12.

28. Genetic sequence according to claim 14, 30 being the amino acid sequence of Fig. 12.

29. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 13.

30. Genetic sequence according to claim 14,  
being the amino acid sequence of Fig. 13.

1 / 20

Oligonucleotides

femX1	TTCMAATCGCGGTCCAGT	213-230
femX2	CAAGAACATGGCAACGAATTACC	913-935
femX3	TGGGTAATTCGTTGCCATGTTCT	937-915
femX4	CCAAGCATCTTCAGCATCTTC	1133-1113
femX5	TTCTTTAACTGTAACTCTGTAAATTCA	1309-1281
femX6	ACATATTTACTTAATTCGTTAAAGAA	290-265
femX7	CAGAAAAATGGTGTTAAAGTAAGATTT	559-585
femX8	AAGAAATCTTACTT TCACACCATTTT	588-562
femX9	AACTCGAAAATAGAACTA	(-43)-(-26)

FIG. 1

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S. haemolyticus	-----t-----g-----a-----a-a-a-t-c-g-tg-caat-a-a-a-taag-c-at-t-t-cc-a-a-atgact-aa
S. hominis	aggagttata gag-t-----g-----a-----a-a-a-t-c-g-tg-caat-a-a-a-taag-c-at-t-t-cc-a-a-atgact-aa
S. aureus	aatagagta atg-t-----g-----a-----a-a-a-t-c-g-tg-caat-a-a-a-taag-c-at-t-t-cc-a-a-atgact-aa
S. epidermidis	999agttatg aag-t-----g-----a-----a-a-a-t-c-g-tg-caat-a-a-a-taag-c-at-t-t-cc-a-a-atgact-aa
S. saprophycicus	aggagttatg aag-t-----g-----a-----a-a-a-t-c-g-tg-caat-a-a-a-taag-c-at-t-t-cc-a-a-atgact-aa
CONSENSUS	-----A-AATGA A-TTAC-AA TTT-AC-GC-A-ACA-TT-G-----T-TAC-GA-----ATG-C--A-AC-C ATTT-AC-CA-A-----G--100
S. haemolyticus	a-c-t-t-ga-g-ag-----aa-taaaca--aa-t-ct--t-t-t-a-a-t-t-a-g--t-g--t-----a-----c-ca-gt-g-a-a-a-a-
S. hominis	a-t-t-gt-a-ag-----tg-gaaact--aa-t-ct--t-t-t-a-a-t-t-a-g--t-g--t-----a-----c-ca-gt-g-a-a-a-a-
S. aureus	c-c-t-gt-a-gc-----tg-aggtat--aa-a-tt--g-a-a-a-c-a-a-g--t-g--t-----a-----c-ca-gt-g-a-a-a-a-
S. epidermidis	a-t-c-at-a-gg-----tg-aggtac--gt-a-tt--t-a-a-t-t-g--t-g--t-----a-----c-ca-gt-g-a-a-a-a-
S. saprophycicus	a-t-t-at-g-aa-----ag-aagtaca--aa-a-cc--a-a-t-t-g--t-g--t-----a-----c-ca-gt-g-a-a-a-a-
CONSENSUS	-A-TA-CA--T-AA--TTGC--A-----GA--C-CA--TAGT-GG-AT-AA-AA-AA--ATAA-GA-G-T-ATTGC-GC-TG--T--T-AC-GC-GT-C200
S. haemolyticus	-a-c-----t-t-t-t-g--c-----t-cc-a-a-a-t-a-t--a-g-t--tag--g--t-t-t-c-tt-----t--g--aaca--
S. hominis	-a-t-t-a--t-----a-tc-t--t-a-c-t--t-a-g-a--caa--g--t-t-t-c-tt-----t--g--aaga--
S. aureus	-g-g-c-g--t-----a-tc-c--t-a-g-t--t-a-g-a--tca--a--c-a-c-tt-----t--g--aaga--
S. epidermidis	-a-a-t-a--t-----c-tc-c--t-a-a-a--a-a-t--taa--g--t-a-t-tt-----t--g--aaga--
S. saprophycicus	-t-t-c-c-g--t-----c-ta-a--t-a-c-a--t-t-g-a--taa--a--c-a-t-tac-----t--g--aaga--
CONSENSUS	C-GT-ATGAA A-T-TT-AA-TA-TTTTATT C-AA--G-GG-CC-GT-AT-GATT-T-A-A-A--AGA-CT-GT-CA-T--TTCTTAA-G A-TT--AA300
S. haemolyticus	g--t-a--c-gc-taa-t gtc-----tg--tcag-t-c--t-tt-ac-a-----t-ta--t-aa--taca-gt--t-t-ta-t
S. hominis	a--t-a--c-ac-aca-t gtc-----tg--acga-a-c--t-tt-gc-t-----tcgt--t-t-ta--taca-ga--t-t-ga-t
S. aureus	a--g-t--a-ac-tcg-t gtc-----cc--acata-c-t--a-tt-ac-a-----ctgt--t-t-c-ga--taca-gt--t-ta-t
S. epidermidis	a--g-a--a-at-taa-t gtt-----tt--aag-t-c--a-c-c-tc-a-----ttga--t-g-a-aa--aact-ga--a-tc-t
S. saprophycicus	a--g-a--a-ac-taa-g cct-----tt--acga-a-t--t-tc-tg-t-----tcgt--t-t-ta--atta-ca--t-g-tc-c
CONSENSUS	-TAT-T-AA-A-A--T-----TATA-T-----T-GA-CC-TA--T--C-TATCAATA-----ATCAT GA-GG-CA--T--G-AA TGC-GG--A-400
S. haemolyticus	-----t-c--t---a-ga-gaagcatc-c--a-t-g-a--tgaa-c--tact-aa-t-----t-ga-taaa-----cc-a-at-t--t-tt-g
S. hominis	-----t-c--t---a-aa-gaaacaat-a--a-a-c-a--cgaa-g--taca-ca-a-----t-aa-atta-----tc-g-tc-t--a-tt-a
S. aureus	-----t-c--t---a-aa-gagtaact-a--a-t-g-a--tact-aa-a-----ccat-aa-a-----t-tg-gcta-----tc-t-at-c--a-gt-g
S. epidermidis	-----a-t--t---g-at-agagagt-a--a-a-a-a--cgaa-a--ccac-aa-a-----tta-ct-c-----c-a-at-t--t-tc-a
S. saprophycicus	-----a-t--t---a-aa-gaaacaac-c--t-a-a-g--tgaa-t--tta-ct-c-----c-aa-actt-----aa-atc-t--t-tt-g
CONSENSUS	GATTGG-T-T-T-GAT-A--T-----T-----CG-T-T-A-C-A-----GG-TTGA-CC--T-----CAAAAT--G-T-CA-TC-GT-TA-500
S. haemolyticus	-----aaaaa t-----cat-t-a-a-ta-at-a-ttga--a-tc-a-c-t-ac-t--t-t-----tc-a-a--t-g-----taag--
S. hominis	-----aagga t-----ctg-t-a-a-tg-at-a-ttga--a-tt-a-c-a-aa-a--t-t-----cc-a-a--t-t-----aaga--
S. aureus	-----aagga t-----cag-a-g-t-ca-ca-t-aaat--g-ac-t-a-a-aa-a--c-g-----ta-a-g--t-t-----aaga--
S. epidermidis	-----gcaaa c-----gtg-t-a-t-tg-tt-a-aaac--g-tt-a-a-a-gt--t-t-----ta-g-a--t-t-----ccgc--
S. saprophycicus	-----gctgg a-----ctg-t-a-a-cg-ac-t-ttgtt--a-tt-a-c-t-ac-a--t-t-----ac-g-a--t-t-----aaga--
CONSENSUS	ATTTA-----AAAA--C--A-GA--T--T-AA-----ATGGAT-G-T--G-AA--G-A-A-AC-AAAAA AGT--A-NA--AATGG-GT-A AACT-----TT600
S. haemolyticus	ct-atcag--a-a-ac-t--a-c-cc-t-----t-----a-----aa-c-aa-g--a-aa-ccaa-----a-a-t--tagt--c--t-t-tc-c
S. hominis	tc-tacta--a-a-at-a--t-t-ca-a-----t-----a-----gt-a-ga-t--a-aa-ttct-----a-a-g--tagt--t--c-t-tc-a
S. aureus	tt-actcg--a-ac-a--a-t-ta-a-----t-----a-----gt-a-at-a--a-ct-ttgt-----c-t-t--caaa-t--c-c-tc-c
S. epidermidis	tt-actcg--a-gt-a--t-a-ta-g-----t-----g-----ct--t-aa-t--a-at-tgca--a-a-a--tagt--t--c-c-ca-a
S. saprophycicus	tt-agtg--t-t-gt-g--a-a-cc-c-----t-----a-----tt-t-aa-a--g-at-tgac--a-a-t--cgat--t--t-t-ta-g
CONSENSUS	--T-----AA GA-GA--T-C C-AT-TT--G--TCATT-ATG GA-GATAC--C-GA--C-AA--G-TT-----GAT-G-G-A-G A---TT-TA--TA-AA--G-700

FIG. 2b

S. haemolyticus	atagac---tc-----tca c-gc-t-a-a--ac-a-a-t--ta-g------g-c a-cgaa---ac-aaat--ac-tg-aact t-a-a-----	800
S. hominis	-ttgatc---tt-----tag a-a-a-a--tc-c-a--aa-a------a-t c-tgaa---c-tc-tgca--ac-tc-gaca t-a-a-----	
S. aureus	-taaat---ac-----cgg t-gt-a-a--tt-a-g------ca-c------a-t a-taaa---c-aa-cgaa--gc-tg-tatt t-a-a-----	
S. epidermidis	-tcaaac---at-----ccg t-tt-a-a--ac-a-c------ta-c------g-t a-agag---c-aa-taat--aa-aa-tgtg c-t-a-----	
S. saprophyticus	-taagat---at-----tcg t-gt-t-c--at-a-t------gg-t------a-t a-aaca---c-aa-ggca--ac-cg-agta t-a-g-----	
CONSENSUS	T-----ATT -AAACA---GT--T-GT-CC--T-GC-T ATAT--A-TT TGATGA-TA--T-----GAA- T--A-----CA -G--A-----T-A-TAAAG	
S. haemolyticus	801	
S. hominis	-tgtt-t-t- -tt-a-a-a--t-t-t-a--c-a-a-a--c-t-t-----g--at-tt-t- -aaaagaa--tc-tg--a-a--t-t-agat- -c-tc-a--	
S. aureus	-ctta-c-c- -tc-a-a-a--t-t-t-a--c-a-a-a--t-t-t-----a--acaat- -aaaata- -tt-ag-c-g--t-aaaa- -a-tg-g--	
S. epidermidis	-ttta-t-t- -gt-a-g--t-t-t-a--c-t-t-t- -a-t-----a--acat-c--gcagat- -ct-ac-c-a--c-tgat- -a-tg-g--	
S. saprophyticus	-ttat-t-t- -tt-a-a-a--c-c-t-g- -c-t-a-a--g-t-t-----a--acat-c--aaagaa- -tt-ag-c-a--c-cgat- -a-tc-g--	
CONSENSUS	tata-t-t- -ag-t-g- -t-a-a-a--a-a-a-a--a-t-----a--gkat-t- -aaagaa- -tt-ag-c-a--c-cgat- -a-cc-a--	
S. haemolyticus	901	
S. hominis	A---AA-AA AGC--T-AA- GA-AT-GA-A AA-G-CC-GA -AA-AAAA- GC-----AA-A A-----AA -T-AA-A- CNA-T-----G A-CA 900	
S. aureus	---at-ag-c--g-ct-aaa aat-ac-gc cg-a-----t--t-----a--t-a--a-gt-t-----c-t-t-----a-t-----t-t-t-t-t	
S. epidermidis	---aa-tg-t--a-ca-cac aac-tc-tt ag-a-----t--t-----a--a-a-t- -t-ga-t-----c-t-t-----a-t-----t-a-a-t-t	
S. saprophyticus	---ga-tg-a--a-gt-aac gcc-ac-ga ag-a-----t--t-----t--c-t-t- -t-gt-t-----c-t-t-----a-t-----t-t-t-t-t	
CONSENSUS	---aa-ta-t--a-ct-aaa act-aa-ca ag-a-----c--t-----c-c-t-t- -t-gc-t-----a-a-t-----g-t-----a-b-t-c-c	
S. haemolyticus	1001	
S. hominis	---aa-ag-t--a-cc-cbg cgt-ac-ga ga-g-----t--t-----g-t-t-t- -a-ct-a-t--a-t-----t-a-----c-t-t-c-t	
S. aureus	AAA--T--A- GA-G--A--- --T-AA-- --A-CATGG- AA-GAATTAC C-AT-TC-GC -G--T-CTT- -T-AT-AATC C-T-TGAAGT -GT-TA-TA-	
S. epidermidis	---a-----a--t-t-t--aa a-ata-a-t--t-a-a-c--t-----ta--t-----aca-----c-----aa-tg- t--gg-t--t g-ta-a-c-	
S. saprophyticus	---a-----g-a--aa a-ata-a-c--c-t-t-a--t-----ag--t-----act-----t-----aa-tg- t--ggc--t g-cc-t-t-	
CONSENSUS	---t-----t--a-a--gc a-tcc-t-t--t-c-a-a--t-----ag--g-----gaa-----t-----at-aa- t--ggc--t g-cc-t-t-	
S. haemolyticus	1101	
S. hominis	---c-----ta-----c---a-t-----gt--a-c-e-t-t- -ttt-a--ca-t-a--t-----a--t-tca-----a--cg-aa-t- -g-t-t-----	
S. aureus	---t-----ga-----t--a--t-a--aa-g-ttcacta -t-t-t-t- -ctt-a--ag-t-a-a--c-----a--tta-----a--tg-aa-t- -a-t-----	
S. epidermidis	---c-----tg-----t--a--a--ca--a-t-t-t- -ctt-t--ag-t-a--c-----a--taca-----a--aa-ta-t- -a-t-----	
S. saprophyticus	---c-----ta-----t--g-c-aag-gt--a-t-t-t- -ctt-c--ag-t-g- -t-----g--c-atg-----c--tg-ta-a--a-c-----	
CONSENSUS	---t-----ta-----t--c-t-----ct--a-t-a-a--caat--tg-t-a--t-----a--tta-----a--tg-ag-a--a-t-----	
S. haemolyticus	1201	
S. hominis	ATT-TATGC --TTAG-GGT -A-TTTA--G A-GA-CC-GA AGATG-GG- GT--T-AA-T T-AAAA-CC -T--ATGC- CA--T-T-G A-TA-GTTGG 1200	
S. aureus	a-c-c-tg-g-----t-t-t- -c-a-a-tt- g-ttcaagt-----aga--c--c-----ga--ta-aaa-ga tttaat--aa g-ggggaat- gacg-atacg	
S. epidermidis	t-t-t-cg-t-----t-a--t-a--aa-g-ttcacta -t-t-t-t- -ctt-a--ag-t-a-a--c-----a--tta-----a--tg-aa-t- -a-t-----	
S. saprophyticus	t-c-ta-t-----a-t- -t-a--tg- t-cgcagca -t-cg-c--c-----ag--ta-agac-ga attttt--gg a-ggggaatt- tcaa-ac---	
CONSENSUS	t-c-ta-t-----t-t- -t-a--aa- g-taacatt-----gaa-c--t-----ac--..aga-at agattt--ag a-ggggaatt- tcta-tt---	
S. haemolyticus	1301	
S. hominis	---t-----t--g-t- -t-g-aa- g-cacaatt-----cga-t-g-----aa--ta-ggat-aa aagaaa--aa c-taatag- agag-actaa	
S. aureus	-GA-TT--T- AAACC-AT-A A-AA-CC-T -TA-----TATA--CA- T-AAAA--T -A-----A-----TA-- -A-----A-----A-----	
S. epidermidis	1329	
S. saprophyticus	-----atga aatttacag agttaac	
CONSENSUS	-----atga aatttacag agttaac	
S. haemolyticus	-----atga aatttacag agttaac	
S. hominis	-----atga aatttacag agttaac	
S. aureus	-----atga aatttacag agttaac	
S. epidermidis	-----atga aatttacag agttaac	
S. saprophyticus	-----atga aatttacag agttaac	
CONSENSUS	-----atga aatttacag agttaac	

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NNNNNNNNNN NNNNAATGA ANTTTACNAA TTTNACGCGN ANAGANTTNN GNNNTNTAC NGANNNNATG NCHNANAGNC ATTTNACNCA NANNNNNGNN
NANTANGANN TNAANTTGC NNAANNNNNN GANNNCANN TAGTNGGNAT NAANAANAAN NATAANGANG TNATTGCGC NTGNTTNTN ACNGCGTNC
CNGTNATGAA ANTTTNAAN TANTTTTATT CNAANNGGG NCCNGTNATN GATTNTNANA ANNNAGANCT NGTNCANTNN TTCTTTAANG ANTTNNNNAA
NTATNTNAAA NANNANNNTN NNTATANT NTNNNNNNNTN GGTNTTANC TNAANNNNAT TNAANNNNAT NGNAANNNGNA ANACNAAAAA AGTNNANAAN AATGGNGTNA AAGTNNNNNTT
GATTGCTNT TNGATNANNT NNNNNNNNTN NNNNTNGAN CCNTANNNTN ANNNNGGNTT NNNNANNGGN TTTGANCCNN TNNNCAAAAT NNGNTNNCAN TCNGTNTAN
ATTTANNNNN NAAAANNCH NANGANNNTN TNAANNNNAT GGATNGNNTN TNAANNNNAT NGNAANNNGNA ANACNAAAAA AGTNNANAAN AATGGNGTNA AAGTNNNNNTT
NNTNNNNNAA GANNNTNC CNATNTTNG CCNTTNGCN GATATACNN CNGANNCNAA NGNTTNNNN GATNGGANG ANNNNTTNTA NTANAANNNGN
TNNNNNNATT NNAAGANN NGTNTNGTN CCNTNGCNT ATATNNANTT TGATGANTAN NTNNNGAAN TNNANNNGA NNGNNANNNN NTNANTAAAG
ANNNAANA AGCNTNAAAN GANATNGANA AANGNCCNGA NAAANAAAAAN GCNNNNAANA ANNNNNNNNA NNTNNAANAN CAANTNNNG CNAANNANCA
AAANTNNAN GANNNNANN NNTNNAAN NNAATATG NNAATATG AANGAATATC CNATNTCNGC NGNTNCTTN NTNATNAATC CNTNTGAAGT NGTNTANTAN
GCNGGTGCGA CNTCNAATNN NTNNNGNCAN TTNGCNGGNA GNTATGCNNT NCAATGGNN ATGATTAANT ATGCNNTNNA NCATNNNATN NANNGTANA
ATTTNTATGG NNTTAGGGT NANTTTANNG ANGANGCNGA AGATGNGGN GTNTNAANT TNAAAAAANG NTNNNATGN GANNNTNTNG ANTANGTTGG
NGANTTNTN AACCNATNA ANAACCNNT NTANNNNNN TATANNNCAN TNAAAAAANT NNAANNANN NNNNNNTANN NANNNNNNNA NNNNNNNNN
NNNNNNATGA AATTACAG AGTTAANN

```

FIG. 3 CONSENSUS SEQUENCE



220 bases	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. hominis</i>
<i>S. aureus</i>	-	-	-
<i>S. epidermidis</i>	17.7	-	-
<i>S. hominis</i>	13.2	16.8	-
<i>S. saprophyticus</i>	17.3	18.6	16.8

Base % ( non apparated ) between the primers bioU1 and bioU3  
FIG4a

FIG. 4b

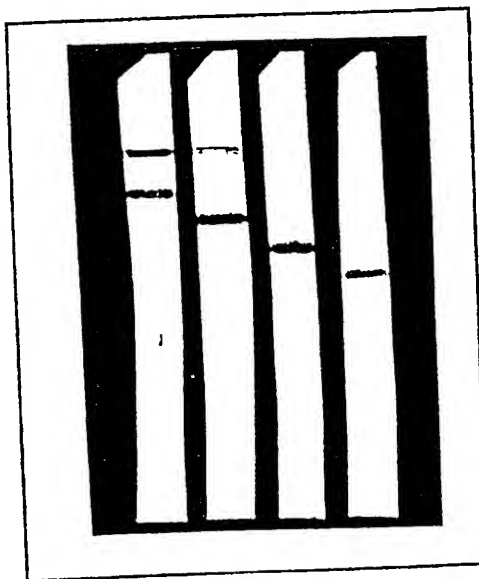
1 : mecA

2: femA Sau

3. femA Sep

4. femA Sho

5. femA Ssa



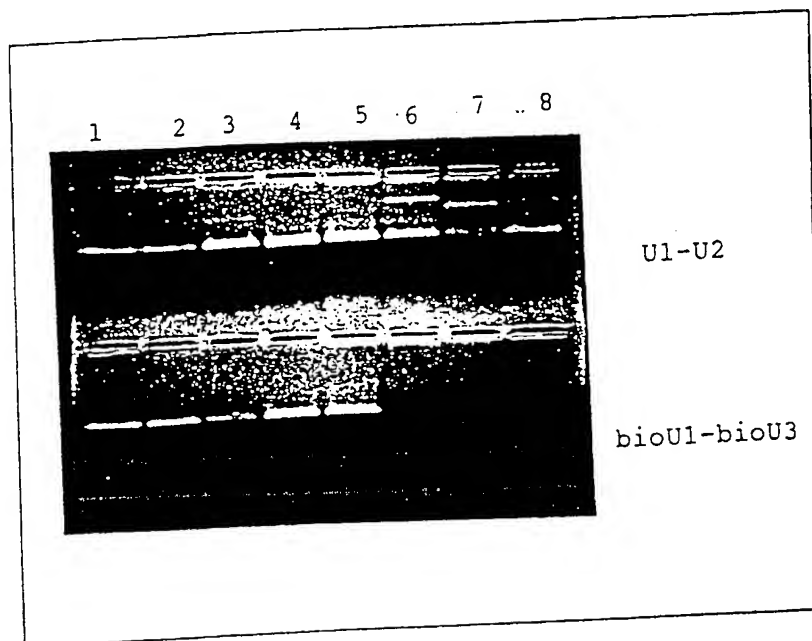


FIG.5

AMPLIFICATION of CNS SPECIES under UNIVERSAL CONDITIONS.

(1) : *S. haemolyticus*

(2) : *S. capitis*

(3) : *S. cohnii*

(4) : *S. xylosum*

(5) : *S. simulans*

(6) : *S. lugdunensis*

(7) : *S. schleiferi*

(8) : *S. warneri*

Th(reaction PCR) = 48°C

.7/20  
S. haemolyticus FIG.6a

10 30 50  
ATAATGAAGTTTACAAATTTAACAGCTACAGAGTTTGGCAATTATACAGATAAGATGCCA  
MetLysPheThrAsnLeuThrAlaThrGluPheGlyAsnTyrThrAspLysMetPro

70 90 110  
TATAGTCATTTACACAAATGACTGAAAACATGAGATGAAAGTTGCAAATAAAACAGAA  
TyrSerHisPheThrGlnMetThrGluAsnTyrGluMetLysValAlaAsnLysThrGlu

130 150 170  
ACTCACTTAGTTGGTATAAAAAATAAAGATAATGAGGTTATTGCAGCCTGCATGTTGACA  
ThrHisLeuValGlyIleLysAsnLysAspAsnGluValIleAlaAlaCysMetLeuThr

190 210 230  
GCAGTACCAGTCATGAAATTTTTTAAGTACTTTTATTCTAACCAGGACCTGTAATTGAT  
AlaValProValMetLysPhePheLysTyrPheTyrSerAsnArgGlyProValIleAsp

250 270 290  
TATGATAATAGAGAGCTTGTTCACTTTTCTTTAATGAGTTAACAAAGTATTTAAACAG  
TyrAspAsnArgGluLeuValHisPhePhePheAsnGluLeuThrLysTyrLeuLysGln

310 330 350  
CATAATTGTCTATATGTTGAGTTGACCCTTATTTACCATATCAATATTTAAATCATGAT  
HisAsnCysLeuTyrValArgValAspProTyrLeuProTyrGlnTyrLeuAsnHisAsp

370 390 410  
GGTGAAATTACAGGTAATGCTGGTAATGATTGGTTCTTTGATAAGATGAAGCATCTCGGA  
GlyGluIleThrGlyAsnAlaGlyAsnAspTrpPhePheAspLysMetLysHisLeuGly

430 450 470  
TTTGAACATGAAGGCTTTACTAAAGGTTTTGATCCGATTAAACAAATCCGATATCATTCT  
PheGluHisGluGlyPheThrLysGlyPheAspProIleLysGlnIleArgTyrHisSer

490 510 530  
GTTTGTAGATTTAAAAATAAAACATCTAAAGATATATTAATGGAATGGATAGTCTACGT  
ValLeuAspLeuLysAsnLysThrSerLysAspIleLeuAsnGlyMetAspSerLeuArg

550 570 590  
AAACGTAATACTAAAAAGTTCAAAAAATGGTGTGAAAGTTAAGTTCTTATCAGAAGAA  
LysArgAsnThrLysLysValGlnLysAsnGlyValLysValLysPheLeuSerGluGlu

610 630 650  
GAACTTCCAATCTCCGTTTCAATTTATGGAAGATACAACCGAAACGAAAGAATTCCAAGAT  
GluLeuProIlePheArgSerPheMetGluAspThrThrGluThrLysGluPheGlnAsp

670 690 710  
AGAGATGATAGTTTCTATTATAATCGCTATAGACATTTCAAAGATCACGTGCTTGTACCA  
ArgAspAspSerPheTyrTyrAsnArgTyrArgHisPheLysAspHisValLeuValPro

8/20

730 750 770  
CTAGCTTATATTAAGTTTGATGAGTACATCGAAGAATTACAAAATGAACGTGAACTTTA  
LeuAlaTyrIleLysPheAspGluTyrIleGluGluLeuGlnAsnGluArgGluThrLeu

790 810 830  
AATAAGATGTTAATAAAGCTTTAAAGATATTGAAAAACGACCAGACAATAAAAGGCA  
AsnLysAspValAsnLysAlaLeuLysAspIleGluLysArgProAspAsnLysLysAla

850 870 890  
TTTAATAAAAAAGAAAATCTTGAAAAACAATTAGATGCCAATCAACAAAAATTAGACGAG  
PheAsnLysLysGluAsnLeuGluLysGlnLeuAspAlaAsnGlnGlnLysLeuAspGlu

910 930 950  
GCTAAAAAATTACAAGCCGAACATGGTAATGAATTACCAATTCAGCAGGTTTCTTCTTT  
AlaLysLysLeuGlnAlaGluHisGlyAsnGluLeuProIleSerAlaGlyPhePhePhe

970 990 1010  
ATTAATCCATTTGAAGTTGTTTATTATGCAGGTGGAACCTTCTAATAAATATAGACATTT  
IleAsnProPheGluValValTyrTyrAlaGlyGlyThrSerAsnLysTyrArgHisPhe

1030 1050 1070  
GCAGGCAGTTATGCTATTCAATGGACAATGATTAACATGCAATTGATCATGGTATTGAT  
AlaGlySerTyrAlaIleGlnTrpThrMetIleAsnTyrAlaIleAspHisGlyIleAsp

1090 1110 1130  
AGATACAATTTCTATGGTATTAGCGGTAATTTTAGTGAAGACGCTGAAGATGTTGGAGTC  
ArgTyrAsnPheTyrGlyIleSerGlyAsnPheSerGluAspAlaGluAspValGlyVal

1150 1170 1190  
ATTAAATTTAAAAAGGTTTCAATGCAGACGTAATTGAGTATGTTGGAGACTTTGTGAAA  
IleLysPheLysLysGlyPheAsnAlaAspValIleGluTyrValGlyAspPheValLys

1210 1230 1250  
CCTATTAACAAACCTTTGTATTAGTGTATAAGACACTCAAAAAGATTAAAAAAGATTT  
ProIleAsnLysProLeuTyrSerValTyrLysThrLeuLysLysIleLysLysArgPhe

1270 1290  
AATTAAAGAGGGGAATAGACGAATATGAAATTTACAGAGTTAAAC  
AsnEndArgGlyGluEndThrAsnMetLysPheThrGluLeuAsn

FIG. 6b

S. lugdunensisFIG. 7a

10 30 50  
ACAGCAAATGAATTCGGTGATTTACAGATCAAATGCCATATAGTCATTTTACTCAAATG  
ThrAlaAsnGluPheGlyAspPheThrAspGlnMetProTyrSerHisPheThrGlnMet

70 90 110  
ACAGGTAACATAATTTAAAAGTTGCCGAAAAACAGAAACACATTTAGTTGGTGTTAA  
ThrGlyAsnTyrAsnLeuLysValAlaGluLysThrGluThrHisLeuValGlyValLys

130 150 170  
AATAATAATAACGAAGTAATTGCAGCATGTTTATTGACAGCTGTACCAGTCATGAAGTTT  
AsnAsnAsnAsnGluValIleAlaAlaCysLeuLeuThrAlaValProValMetLysPhe

190 210 230  
TTTAAATACTTTTACAGCAATAGAGGCCAGTTATAGATTATGCTAACCAAGAACTTGTA  
PheLysTyrPheTyrSerAsnArgGlyProValIleAspTyrAlaAsnGlnGluLeuVal

250 270 290  
CATTTTTCTTTAATGAGCTAACTAAATATTTAAAAAAGTATAACTGTCTCTATGTCCGC  
HisPhePhePheAsnGluLeuThrLysTyrLeuLysLysTyrAsnCysLeuTyrValArg

310 330 350  
ATAGATCCATACTTACCTTATCAATATAGAGACCATGACGGTAATATAACGGCAAATGCT  
IleAspProTyrLeuProTyrGlnTyrArgAspHisAspGlyAsnIleThrAlaAsnAla

370 390 410  
GGCAATGATTGGTTTTTCAATAAAATGGAACAACTCGGATACCATCATGATGGCTTTACA  
GlyAsnAspTrpPhePheAsnLysMetGluGlnLeuGlyTyrHisHisAspGlyPheThr

430 450 470  
ACAGGATTTGATCCAATATTACAAATCAGATTCCATTCTATTCTTAATTTAAAGGATAAG  
ThrGlyPheAspProIleLeuGlnIleArgPheHisSerIleLeuAsnLeuLysAspLys

490 510 530  
ACAGCTAAAGATGTTTTAAATAATATGGATAGTTTACGTAAAAGAAATACCAAAAAAGT  
ThrAlaLysAspValLeuAsnAsnMetAspSerLeuArgLysArgAsnThrLysLysSer

550 570 590  
TCAAAAAATGGAGTCAAAGTAAAGTTCCTTACTGAAGAAGAACTACCTATCTTTTCGTTCA  
SerLysAsnGlyValLysValLysPheLeuThrGluGluGluLeuProIlePheArgSer

610 630 650  
TTTATGGAGCAGACGTCAGAATCTAAAGAATTCTCTGATAGAGACGACCAATTTTATTAC  
PheMetGluGlnThrSerGluSerLysGluPheSerAspArgAspAspGlnPheTyrTyr

670 690 710  
AATCGGTTTTAAGTACTATAAAGATAGGGTGCTTGTGCCTCTAGCATATTTAAAAATTTGAT  
AsnArgPheLysTyrTyrLysAspArgValLeuValProLeuAlaTyrLeuLysPheAsp

10/20

730 750 770  
GAATATATAGAAGAACTAACGAATGAACGACAACTTTAGAAAAAGATTTAGGCAAAGCA  
GluTyrIleGluGluLeuThrAsnGluArgGlnThrLeuGluLysAspLeuGlyLysAla

790 810 830  
CTTAAAGACATTGAGAAACGACCAGATAACAAAAAGCTTATAATAACGAGACAACCTA  
LeuLysAspIleGluLysArgProAspAsnLysLysAlaTyrAsnLysArgAspAsnLeu

850 870 890  
CAACAACAACCTCGATGCCAATCAACAAAAGTTAAATGAGGCTAATCAGTTACAAGCGGAA  
GlnGlnGlnLeuAspAlaAsnGlnGlnLysLeuAsnGluAlaAsnGlnLeuGlnAlaGlu

910 930 950  
CACGGTAATGAGTTACCTATCTCTGCCGTTTCTTTATTATTAAATCCGTTTGAAGTTGTA  
HisGlyAsnGluLeuProIleSerAlaGlyPhePheIleIleAsnProPheGluValVal

970 990 1010  
TACTACGCTGGAGGTACCGCTAATAAATATCGTCATTTTGCAGGTAGTTACGCGGTTTCAG  
TyrTyrAlaGlyGlyThrAlaAsnLysTyrArgHisPheAlaGlySerTyrAlaValGln

1030 1050 1070  
TGGACTATGATTAACCTATGCTATCGAACACGGCATAGACAGATATAATTTCTACGGCATT  
TrpThrMetIleAsnTyrAlaIleGluHisGlyIleAspArgTyrAsnPheTyrGlyIle

1090 1110 1130  
AGTGGAACCTTCTCAGATGATGCTGAAGACGCAGGTGTCATTCGCTTTAAAAAAGGTTAT  
SerGlyAsnPheSerAspAspAlaGluAspAlaGlyValIleArgPheLysLysGlyTyr

1150 1170 1190  
GGTGCAGAAGTGATTGAATACGTTGGTGATTTTGTAAAACCTATAAATAAACCTATGTAT  
GlyAlaGluValIleGluTyrValGlyAspPheValLysProIleAsnLysProMetTyr

1210 1230 1250  
AAACTTTATTTCAGTGTTAAAACGAATTCAAAATAAGCTATAGAGGAGAATGGATTAAATTA  
LysLeuTyrSerValLeuLysArgIleGlnAsnLysLeuEndArgArgMetAspEndLeu

1270  
TGAAATTTACAGAGTTTAAC  
EndAsnLeuGlnSerLeu

FIG. 7b

11/20  
S. xylosusFIG. 8a

10 30 50  
ACGCAAAAGAGTTTGGGTGCATTTTCAGATAAAATGCCAAATAGCCATTTACGCAAATG  
ThrGlnLysSerLeuGlyAlaPheSerAspLysMetProAsnSerHisPheThrGlnMet

70 90 110  
GTAGGGAATTATGAATTGAAAATTGCAGAAAGTACTGAAACACATTAGTAGGTATAAAA  
ValGlyAsnTyrGluLeuLysIleAlaGluSerThrGluThrHisLeuValGlyIleLys

130 150 170  
AACAAATGATAATGAAGTCATTGCAGCTTGTTTATTAAGTGCAGTACCAGTAATGAAATTC  
AsnAsnAspAsnGluValIleAlaAlaCysLeuLeuThrAlaValProValMetLysPhe

190 210 230  
TTTAAGTATTTTATACTAATAGAGGTCCGGTTATAGATTTTGAAAATAAGAATTAGTG  
PheLysTyrPheTyrThrAsnArgGlyProValIleAspPheGluAsnLysGluLeuVal

250 270 290  
CATTACTTTTTCAATGAATCTCTAAATATGTGAAAAACATAATGCGCTTTATTTAAGA  
HisTyrPhePheAsnGluLeuSerLysTyrValLysLysHisAsnAlaLeuTyrLeuArg

310 330 350  
GTTGATCCTTATTTAGCATATCAATACCGTAATCATGATGGTGAGGTATTGGAAAATGCA  
ValAspProTyrLeuAlaTyrGlnTyrArgAsnHisAspGlyGluValLeuGluAsnAla

370 390 410  
GGACATGATTGGATTTTCGATAAAATGAAGCAGCTTGGATATAAACACCAAGGATTTTAA  
GlyHisAspTrpIlePheAspLysMetLysGlnLeuGlyTyrLysHisGlnGlyPheLeu

430 450 470  
ACTGGTTTCGATTCAATTATTCAAATTAGGTTCCACTCTGTACTGGATTTAGTAGGTAAA  
ThrGlyPheAspSerIleIleGlnIleArgPheHisSerValLeuAspLeuValGlyLys

490 510 530  
ACTGCTAAAGATGTACTAAATGGTATGGATAGTTTACGTAAACGTAATACTAAAAAAGTA  
ThrAlaLysAspValLeuAsnGlyMetAspSerLeuArgLysArgAsnThrLysLysVal

550 570 590  
CAAAAAATGGCGTGAAAGTAAGGTTCTTAAGGAAGATGAGTTGCCAATTTCCGTTCA  
GlnLysAsnGlyValLysValArgPheLeuArgGluAspGluLeuProIlePheArgSer

610 630 650  
TTCATGGAAGATACATCTGAACTAAAGACTTTGACGATAGAGACGATGGCTTTTACTAC  
PheMetGluAspThrSerGluThrLysAspPheAspAspArgAspAspGlyPheTyrTyr

670 690 710  
AATAGATTAAGGTATTATAAAGATCGCGTATTAGTACCTCTAGCTTATATGGATTTCAAT  
AsnArgLeuArgTyrTyrLysAspArgValLeuValProLeuAlaTyrMetAspPheAsn

730 750 770  
GAATATATTGAAGAATTGCAAGCTGAACGTGAGGTGTTAAGCAAAGATATCAATAAAGCA  
GluTyrIleGluGluLeuGlnAlaGluArgGluValLeuSerLysAspIleAsnLysAla

790 810 830  
GTAAAAGATATCGAGAAAAGACCTGAAAATAAAAAAGCATATAATAAAAAAGATAATCTA  
ValLysAspIleGluLysArgProGluAsnLysLysAlaTyrAsnLysLysAspAsnLeu

850 870 890  
GAGAAACAACCTTATAGCGAATCAACAAAAAATTGATGAAGCTAAACTCTACAAGAGAAG  
GluLysGlnLeuIleAlaAsnGlnGlnLysIleAspGluAlaLysThrLeuGlnGluLys

910 930 950  
CATGGTAACGAACCTACCAATCTCAGCAGCATATTTTCATCATTAAACCCTTATGAAGTAGTG  
HisGlyAsnGluLeuProIleSerAlaAlaTyrPheIleIleAsnProTyrGluValVal

970 990 1010  
TATTATGCGGGTGGAACGTCAAATGAGTTTATGACATTTTGCTGGTAGTTATGCCATTCAA  
TyrTyrAlaGlyGlyThrSerAsnGluPheArgHisPheAlaGlySerTyrAlaIleGln

1030 1050 1070  
TGGAAGATGATTAACCTATGCTATTGACCATAATATTGATAGATATAATTTTTATGGAATT  
TrpLysMetIleAsnTyrAlaIleAspHisAsnIleAspArgTyrAsnPheTyrGlyIle

1090 1110 1130  
AGTGGTCATTTTACAGAAGATGCAGAAGATGCCGGTGTAGTTAAATTTAAAAAAGGATTT  
SerGlyHisPheThrGluAspAlaGluAspAlaGlyValValLysPheLysLysGlyPhe

1150 1170 1190  
AATGCGGATGTAGTGGAATATGTTGGTGATTTTATTAAACCAATCAATAAACCAATGTAC  
AsnAlaAspValValGluTyrValGlyAspPheIleLysProIleAsnLysProMetTyr

1210 1230 1250  
AAAATTTATACGACATTAAAGAAAATTAAAGATAAAAAGAAATAAACATTTAATAGAAGG  
LysIleTyrThrThrLeuLysLysIleLysAspLysLysLysEndThrPheAsnArgArg

1270 1290  
GAACTAAGCTAGAATGAAATTTACAGAGTTAAACC  
GluLeuSerEndAsnGluIleTyrArgValLys

FIG.8b



S. capitis FIG. 9a

10 30 50  
ACAGCTAAAGAATTTAGTGACTTTACTGATCAAATGCCTTATAGCCATTTTACTCAGATG  
ThrAlaLysGluPheSerAspPheThrAspGlnMetProTyrSerHisPheThrGlnMet

70 90 110  
GAAGGTAATTATGAACTTAAAGTTGCTGAAGGTACGGATTACATCTCGTAGGAATTAA  
GluGlyAsnTyrGluLeuLysValAlaGluGlyThrAspSerHisLeuValGlyIleLys

130 150 170  
AATAATGACAACCAAGTGATTGCAGCATGTTTATTAAGTCTGTACCTGTAATGAAAATT  
AsnAsnAspAsnGlnValIleAlaAlaCysLeuLeuThrAlaValProValMetLysIle

190 210 230  
TTTAAATATTTTTACTCAAATCGCGGGCCAGTGATTGATTATGATAATAAAGAGCTTGTT  
PheLysTyrPheTyrSerAsnArgGlyProValIleAspTyrAspAsnLysGluLeuVal

250 270 290  
CACTTTTCTTTAATGAATTAAGTAAATATGTAAAAAGCATAATTGTCTTTATCTAAGA  
HisPhePhePheAsnGluLeuSerLysTyrValLysLysHisAsnCysLeuTyrLeuArg

310 330 350  
GTTGACCCTTATCTTCCTTATCAATACTTAAATCATGACGGTGAAATTATTGGAAATGCT  
ValAspProTyrLeuProTyrGlnTyrLeuAsnHisAspGlyGluIleIleGlyAsnAla

370 390 410  
GGCCATGATTGGTTTTTCAATAAGATGGAAGAATTAGGATTTGAACATGAAGGCTTTCAT  
GlyHisAspTrpPhePheAsnLysMetGluGluLeuGlyPheGluHisGluGlyPheHis

430 450 470  
AAAGGCTTCCATCCTATCTTACAAGTAAGATATCATTGAGTTTTAGATTTAAAGATAAA  
LysGlyPheHisProIleLeuGlnValArgTyrHisSerValLeuAspLeuLysAspLys

490 510 530  
ACGGCTAAAGATGTACTCAAAGGAATGGATAGTTTAAAGAAAGCGTAATACTAAGAAAGTA  
ThrAlaLysAspValLeuLysGlyMetAspSerLeuArgLysArgAsnThrLysLysVal

550 570 590  
CAAAAAATGGTGTCAAAGTCCGTTTCCTATCCGAAGATGAATTACCTATCTTTAGATCA  
GlnLysAsnGlyValLysValArgPheLeuSerGluAspGluLeuProIlePheArgSer

610 630 650  
TTTATGGAAGATACTACAGAAACGAAAGAGTTCGCCGATAGAGATGATAGTTTCTATTAT  
PheMetGluAspThrThrGluThrLysGluPheAlaAspArgAspAspSerPheTyrTyr

14/20

670 690 710  
AATCGATTAAATACTTTAAAGATAGAGTATTAGTACCATTAGCATATGTTGACTTCGAT  
AsnArgLeuLysTyrPheLysAspArgValLeuValProLeuAlaTyrValAspPheAsp

730 750 770  
GAGTATATTGAAGAACTTAATAATGAAAGAGATGTTCTTAATAAGATTAAATAAGGCG  
GluTyrIleGluGluLeuAsnAsnGluArgAspValLeuAsnLysAspLeuAsnLysAla

790 810 830  
CTCAAAGATATTGAGAAGAGACCTGATAATAAGAAAGCTTATAACAAAAGAGATAATCTT  
LeuLysAspIleGluLysArgProAspAsnLysLysAlaTyrAsnLysArgAspAsnLeu

850 870 890  
CAACAACAATTAGATGCAAAATCAACAAAAAATTGATGAAGCTAAAACTTACAACAAGAA  
GlnGlnGlnLeuAspAlaAsnGlnGlnLysIleAspGluAlaLysAsnLeuGlnGlnGlu

910 930 950  
CATGGTAATGAATTACCTATTTTCAGCTGGATATTTCTTCATTAATCCGTTTGAAGTTGTT  
HisGlyAsnGluLeuProIleSerAlaGlyTyrPhePheIleAsnProPheGluValVal

970 990 1010  
TATTACGCAGGTGGCACATCGAATCGTTATCGTCACTATGCCGGAAGTTATGCAATTCAA  
TyrTyrAlaGlyGlyThrSerAsnArgTyrArgHisTyrAlaGlySerTyrAlaIleGln

1030 1050 1070  
TGGAAAATGATAAACTATGCTTTAGAACATGGAATTAACCGTTATAATTTTTATGGAGTT  
TrpLysMetIleAsnTyrAlaLeuGluHisGlyIleAsnArgTyrAsnPheTyrGlyVal

1090 1110 1130  
AGTGGGGACTTCAGTGAAGACGCTGAAGATGTAGGAGTAATTAAGTTCAAAAAAGGCTAT  
SerGlyAspPheSerGluAspAlaGluAspValGlyValIleLysPheLysLysGlyTyr

1150 1170 1190  
AATGCTGATGTTATTGAATATGTAGGTGATTTTATCAAGCCAATCAATAAACCTATGTAT  
AsnAlaAspValIleGluTyrValGlyAspPheIleLysProIleAsnLysProMetTyr

1210 1230 1250  
GCAATCTATAACGCACTTAAAAAGTTAAAGAAATAGATTTTTTTACCAACCCAATTATCT  
AlaIleTyrAsnAlaLeuLysLysLeuLysLysEndIlePheLeuProThrGlnLeuSer

1270  
AATTATGAAATTTACAGAGTTAA  
AsnTyrGluIleTyrArgVal

FIG. 9b

FIG. 10a

10 30 50  
ACGACGGCTGAATTTGGTGCGTTTACAGATCAAATGCCATATAGCCATTTACGCAAATG  
ThrThrAlaGluPheGlyAlaPheThrAspGlnMetProTyrSerHisPheThrGlnMet

70 90 110  
GTAGGGAACCTATGAATTAAAGGTTGCTGAAGGTGTTGAAACACATCTTGTCGGCATTAA  
ValGlyAsnTyrGluLeuLysValAlaGluGlyValGluThrHisLeuValGlyIleLys

130 150 170  
GATAACAACAATAACGTACTAGCAGCATGTTTACTGACAGCAGTGCCAGTAATGAAGTTT  
AspAsnAsnAsnValLeuAlaAlaCysLeuLeuThrAlaValProValMetLysPhe

190 210 230  
TTTAAATATTTTATTCAAACCGCGGACCAGTCTGACTACGAAAATAAAGAGCTCGTT  
PheLysTyrPheTyrSerAsnArgGlyProValMetAspTyrGluAsnLysGluLeuVal

250 270 290  
CATTTCTTTTTTAATGAACCTTCAAATATGTTAAGAAATATCACGCATTGTATTTGAGA  
HisPhePhePheAsnGluLeuSerLysTyrValLysLysTyrHisAlaLeuTyrLeuArg

310 330 350  
GTAGACCCCTTATTACCAATGTTAAAGCGAAACCATGATGGTGAAGTGATTGAAAGATAC  
ValAspProTyrLeuProMetLeuLysArgAsnHisAspGlyGluValIleGluArgTyr

370 390 410  
GGCAGTGACTGGTTTTTGTATAAAATGGCTGAATTAACTTTGAACATGAAGGTTTCACA  
GlySerAspTrpPhePheAspLysMetAlaGluLeuAsnPheGluHisGluGlyPheThr

430 450 470  
ACTGGGTTTGATACAATAAGGCAAATTCGTTTTTCATTCTGTGCTCGATGTTGAAAATAAA  
ThrGlyPheAspThrIleArgGlnIleArgPheHisSerValLeuAspValGluAsnLys

490 510 530  
ACATCAAAAAGACATCTTAAATCAAATGGATAATTTAAGGAAAAGAAATACGAAAAAGTA  
ThrSerLysAspIleLeuAsnGlnMetAspAsnLeuArgLysArgAsnThrLysLysVal

550 570 590  
CAGAAAAATGGTGTGAAAGTCCGCTATCTAAACGAAGATGAATTACATATTTCCGTTTCG  
GlnLysAsnGlyValLysValArgTyrLeuAsnGluAspGluLeuHisIlePheArgSer

610 630 650  
TTTATGGAAGATACATCTGAAACAAAAGATTTTGTAGATAGAGATGACGATTTTATTAT  
PheMetGluAspThrSerGluThrLysAspPheValAspArgAspAspPheTyrTyr

670 690 710  
CATCGTATGAAATACTATAAAGATCGTGTCCGCGTACCACTAGCGTATATTGATTTTAAT  
HisArgMetLysTyrTyrLysAspArgValArgValProLeuAlaTyrIleAspPheAsn

730 750 770  
GCATATTTAGCAGAGCTCAACACTGAAGCGCAAGACTTTAAAAAGAAATTGCAAAAGCA  
AlaTyrLeuAlaGluLeuAsnThrGluAlaGlnAspPheLysLysGluIleAlaLysAla

790 810 830  
GATAAAGACATCGACAAGCGTCCTGAAAATCAGAAAGCCATAAATAAAAAAGAAAAATTTA  
AspLysAspIleAspLysArgProGluAsnGlnLysAlaIleAsnLysLysLysAsnLeu

850 870 890  
GAGCAACAACCTAGAAGCGAATCAAGCTAAAATAAAAGAAGCAGAAACATTGCAACTTAAA  
GluGlnGlnLeuGluAlaAsnGlnAlaLysIleLysGluAlaGluThrLeuGlnLeuLys

910 930 950  
CACGGTGACACATTACCGATTTCGGCTGGATTCTTTATTATTAATCCATTTGAGGTTGTT  
HisGlyAspThrLeuProIleSerAlaGlyPhePheIleIleAsnProPheGluValVal

970 990 1010  
TATTATGCAGGCGGCACAGCAAACGAATTTCGTCATTTTGCTGGAAGCTACGCAGTGCAA  
TyrTyrAlaGlyGlyThrAlaAsnGluPheArgHisPheAlaGlySerTyrAlaValGln

1030 1050 1070  
TGGGAAATGATTAATTATGCGATTGATTATCAAATCCAAGATATAACTTTTATGGCATT  
TrpGluMetIleAsnTyrAlaIleAspTyrGlnIleProArgTyrAsnPheTyrGlyIle

1090 1110 1130  
AGTGGTGATTTTTCAGAAGATGCAGAAGATGCAGGTGTGATAAAATTTAAAAAGGCTAT  
SerGlyAspPheSerGluAspAlaGluAspAlaGlyValIleLysPheLysLysGlyTyr

1150 1170 1190  
AATGCAGAAGTAATAGAATATGTCGGTGATTTTATTAAGCCTATAAACAACCTGCCTAT  
AsnAlaGluValIleGluTyrValGlyAspPheIleLysProIleAsnLysProAlaTyr

1210 1230 1250  
ACAGTCTACTTAAATTAAGCAATTAAAAGACAAGATAAAAGATAAGATATAGCAAAG  
ThrValTyrLeuLysLeuLysGlnLeuLysAspLysIleLysArgEndAspIleAlaLys

1270 1290  
AGAAGGGGATTTATTGGTATGAAATTTACAGAGTTAA  
ArgArgGlyPheIleGlyMetLysPheThrGluLeu

FIG.10b

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10 30 50  
ACACTGGAATTTGAAGCTTTTACAAATAAAATGCCGTACGCGCATTTTACACAAGCAGTA  
ThrLeuGluPheGluAlaPheThrAsnLysMetProTyrAlaHisPheThrGlnAlaVal

70 90 110  
GGTAATTATGAATTAACATCTGAAGGTACTTCAACACATTTAGTAGGGGTCAAAGAT  
GlyAsnTyrGluLeuLysThrSerGluGlyThrSerThrHisLeuValGlyValLysAsp

130 150 170  
AATCAAGGTGAAGTATTAGCTGCGTGTCTGTTAACAAGGTACCAGTTATGAAGAAATTT  
AsnGlnGlyGluValLeuAlaAlaCysLeuLeuThrSerValProValMetLysLysPhe

190 210 230  
AATTACTTTTACTCAAATAGAGGACCAGTAATGGATTATGACAACAAAGAACTTGTTGAC  
AsnTyrPheTyrSerAsnArgGlyProValMetAspTyrAspAsnLysGluLeuValAsp

250 270 290  
TTTTTCTTTAAAGAAATCGTGAGCTATTTAAAAAGTTATAAAGGATTATTCTTTAGAATC  
PhePhePheLysGluIleValSerTyrLeuLysSerTyrLysGlyLeuPhePheArgIle

310 330 350  
GATCCTTACTTGCCATATCAACTAAGAGATCATGATGGCAATATTAATAAATCATTCAAC  
AspProTyrLeuProTyrGlnLeuArgAspHisAspGlyAsnIleLysLysSerPheAsn

370 390 410  
CGTGATGGTTAATTAAACAATTTGAATCATTAGGTTATGAACACCAAGGCTTCACAACT  
ArgAspGlyLeuIleLysGlnPheGluSerLeuGlyTyrGluHisGlnGlyPheThrThr

430 450 470  
GGTTTCCACCCAATACATCAAATTAGATGGCATTCTGTACTTGATTTAGAAAGTATGGAC  
GlyPheHisProIleHisGlnIleArgTrpHisSerValLeuAspLeuGluSerMetAsp

490 510 530  
GAAAAGACGCTCATCAAGAACATGGACAGTTTAAGAAAAAGAAATACTAAAAAGTTCAA  
GluLysThrLeuIleLysAsnMetAspSerLeuArgLysArgAsnThrLysLysValGln

550 570 590  
AAAAATGGTGTTAAAGTTCGTTTCTATCTAAAGATGAAATGCCGATATCCGTCAATTT  
LysAsnGlyValLysValArgPheLeuSerLysAspGluMetProIlePheArgGlnPhe

610 630 650  
ATGGAAGATACTACAGAGAAGAAAGATTTCAACGATCGTGGCGATGACTTCTATTACAAT  
MetGluAspThrThrGluLysLysAspPheAsnAspArgGlyAspAspPheTyrTyrAsn

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670 690 710  
AGATTAAATACTTTGAAAATGTAAAGATTCCTTTAGCATATATAGACTTTGAAACTTAC  
ArgLeuLysTyrPheGluAsnValLysIleProLeuAlaTyrIleAspPheGluThrTyr

730 750 770  
ATTCCACAATTAGAAAAAGAACATGAACAATACAACAAAGATATTGCAAAAGCTGAAAAA  
IleProGlnLeuGluLysGluHisGluGlnTyrAsnLysAspIleAlaLysAlaGluLys

790 810 830  
GATTTAGAAAAGAAACCAGATAATCAAAAAACGATTAATAAAATAGACAACTTAAACAA  
AspLeuGluLysLysProAspAsnGlnLysThrIleAsnLysIleAspAsnLeuLysGln

850 870 890  
CAAAGAGAAGCAAATGAAGCTAAATTAGAAGAAGCACTTCAACTACAACAAGAACATGGT  
GlnArgGluAlaAsnGluAlaLysLeuGluGluAlaLeuGlnLeuGlnGlnGluHisGly

910 930 950  
GATACATTACCAATAGCAGCTGGTTTCTTTATTATTAAATCCATTGAAGTTGTATATTAT  
AspThrLeuProIleAlaAlaGlyPhePheIleIleAsnProPheGluValValTyrTyr

970 990 1010  
GCAGGTGGTTCATCGAATGAATATCGTCACTTTGCAGGTAGTTATGCAATTCAGTGGGAA  
AlaGlyGlySerSerAsnGluTyrArgHisPheAlaGlySerTyrAlaIleGlnTrpGlu

1030 1050 1070  
ATGATTAAATACGCGTTAGATCACAAACATTGACCGTTATAACTTCTATGGTATCAGCGGA  
MetIleLysTyrAlaLeuAspHisAsnIleAspArgTyrAsnPheTyrGlyIleSerGly

1090 1110 1130  
GACTTCTCAGAAGATGCACCTGATGTTGGCGTTATTAAATTTAAAAAGGTTACAATGCA  
AspPheSerGluAspAlaProAspValGlyValIleLysPheLysLysGlyTyrAsnAla

1150 1170 1190  
GATGTTTATGAATATATTGGTGATTTTCGTTAAACCAATTAATAAACAGCGTACAAAGCA  
AspValTyrGluTyrIleGlyAspPheValLysProIleAsnLysProAlaTyrLysAla

1210 1230 1250  
TATACAACACTAAAAAAGTATTAAAAAATAAATGATTTTCAGTAAGAGAGGAATTTAG  
TyrThrThrLeuLysLysValLeuLysLysEndMetIlePheSerLysArgGlyIleEnd

1270  
ATAATATGAAATTTACAGAGTTAA  
IleIleEndAsnLeuGlnSerEnd

FIG.11b

*Staphylococcus hominis*

taaaattttaaaattagtcactcaaaattaaagattctaaattaggaggtatagagataatgaagttttacaaattttacagcttacagaatttggcg 100  
M K F T N L T A T E F G D  
ATTTTACTGAAANAATGCCATATAGCCATTTTACACAGATGACTGAANAATATGAGTTTAAAGTTGCTGAGANAATGAACTCAATTTAGTAGGAATTAA 200  
F T E K M P Y S H F T Q M T E N Y E L K V A E K T E T H L V G I K  
AATAAAGATAATGAAGTCATTGCTGCTTGTATGCTAACTGCTGACCGTTATGANAATTTTAAATATTTTATTCAAATCGTGGTCCAGTCATTGAT 300  
N K D N E V I A A C M L T A V P V M K I F K Y F Y S N R G P V I D  
TATGAAAACAAAGAACTCGTTCACTTTTCTTTAAACGAATTAAGTAATAATTTAAACAAACACATGTTTATATAGTACGTATAGACCCTTATTTGCCCTT 400  
Y E N K E L V H F F F N E L S K Y L K Q Q H C L Y V R I D P Y L P Y  
ATCNAATATCGTAATCATGTGATATACAGGAATGCTGGGAATGATTTGTTCTTCGATAAATGAACAAATTAGGATATCAACACGAAGSGTTTAC 500  
Q Y R N H D G D I T G N A G N D W F F D K M K Q L G Y Q H E G F T  
AACAGGTTTGTNCCNATATTACAAATTCGGTTCCATTCCAGTTTAAATTTTAAAGGATAAATCTGCTAAAGATGTTTAAATGGAATGGATAGTTTACGA 600  
T G F D P I L Q I R F H S V L N L K D K T A K D V L N G M D S L R  
AANAAGAAATCTAAANAAGTCCNAANAATGGTGTAAAGTAAGATTCTTACTAAGAGAAATTAACCTATTTTCAGATCATTTTATGGAAGATACATCAG 700  
K R N T K K V Q K N G V K V R F L T K E E L P I F R S F M E D T S E  
AGACTAAAGAAATTTTCTGATAGAGAGGATAGTTTACTATAATCGATTGATCAATTTTAAAGATAGATATTAGTACCTCTCGCATATATANAATTTGA 800  
T K E F S D R E D S F Y Y N R F D H F K D R V L V P L A Y I K F D  
TGNATATCTTGAGAACTTCATGCAGAACGTCAGACATTAATAAAGACTTAAACAAAGCTCTTAAAGATATTTGANAACAGCACCATTAACAAAAGCA 900  
E Y L E E L H A E R Q T L N K D L N K A L K D I E K R P D N K K A  
CAAAATAAANAANAATTTAGAACAGCAATTAAGCAAAATGAGCAAAAATGATGAAGCAACACAACCTTCAATTAGAACATGGTAACGATTAACCA 1000  
Q N K K I N L E Q Q L K A N E Q K I D E A T Q L Q L E H G N E L P I  
TATCTGCTGGATCTCTTTTATTATCCATTTGAAGTTGTATATTATGAGGTGGAACCTCAAAATTAATATAGACACTTCGCTGGAAGTTATGCAGTTCA 1100  
S A G F F F I N P F E V V Y Y A G G T S N K Y R H F A G S Y A V Q  
ATGGACTATGATTAATTAATGCAATTCATCGCATTCACCGTTATATTTTATGGATATGCTCATTTTACAGATGATGCTGAAGATGCGAGGTGTT 1200  
W T M I N Y A I D H G I D R Y N F Y G I S G H F T D D A E D A G V  
GTAAAAATTTAAAGGATTTAATGCGATGTAATTTGGTATTTCTGTTAAACCTATAAATAACCAATGTATTCACCTATATACACACTTA 1300  
V K F K K G F N A D V I E Y V G D F V K P I N K P M Y S L Y T T L K  
NAAAAATTAANAAGAGATTGAATTAAGagggggaatagtgagaa 1343  
K I K K R L N ///

FIG.12

FIG. 13.